

ORIGINAL ARTICLE

Role of CD4⁺CD25^{high+}FOXP3⁺ Regulatory T Cells in Psoriasis

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Background: CD4⁺CD25^{high+} regulatory T cells (Tregs) are considered to be of vital importance for maintaining immunologic self-tolerance and preventing autoimmune diseases. These cells have been found to be deficient in skin lesions and in the peripheral blood of patients with psoriasis.

Objective: To investigate the role of Tregs in the pathogenesis of psoriasis and to evaluate the changes in Tregs in relation to the severity and the clinical course of psoriasis.

Methods: Immunohistochemistry (CD3, 4, 8, 79 and FOXP3) was performed in 22 psoriatic patients compared to 5 normal controls. Flow cytometry (CD3, 4, 8, 25 and FOXP3) was performed in 18 psoriatic patients and 8 normal volunteers and reverse transcriptase polymerase chain reaction (*foxp3* mRNA) was performed in 8 psoriasis patients. **Results:** An increase in the FOXP3⁺ cell fraction was detected in the lesional psoriatic skin irrespective of the severity of psoriasis as compared with the normal skin. However, a decrease in FOXP3⁺ cells was observed in the samples obtained from psoriasis of 'acute course'. FOXP3⁺ Treg populations in the blood of the 'acute course' psoriasis was not different compared to that of 'chronic course' psoriasis and normal controls. **Conclusion:** The deficiency of FOXP3⁺ Tregs in the lesional psoriatic skin might be responsible for the exacerbation of psoriasis. (Ann Dermatol 22(4) 397~403, 2010)

-Keywords-

CD4⁺CD25^{high+} regulatory T cells, FOXP3, Psoriasis

INTRODUCTION

CD4⁺CD25^{high+} regulatory T cells (Tregs) were identified in lesional psoriatic plaques. Tregs are a recognized subset of regulatory T cells which actively suppress self-reactive T cells and maintain immunologic self-tolerance. Phenotypically, Tregs express high levels of FOXP3, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), certain members of toll-like receptors, CD103 ($\alpha_E\beta_7$ integrin) and glucocorticoid-induced TNF receptor family-related gene (GITR)¹⁻⁴. Tregs also express lymphocyte activation gene-3, programmed death receptor-1 and neurophilin on their surface⁵. Among these, Tregs were seen to be controlled by the transcription factor, FOXP3, thus it is considered to be most crucial.

Tregs have been reported to be decreased in a variety of diseases with immune dysregulation, including transplantation rejection⁶ and graft versus host disease^{7,8}, neoplastic conditions including breast adenocarcinoma, adenocarcinoma of the colon and lung cancer⁹⁻¹¹, and various other autoimmune diseases.

The purpose of this study was to investigate the role of Tregs in the pathogenesis of psoriasis and to evaluate the changes in Tregs in relation to the severity and the clinical course of psoriasis.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board

Received March 29, 2010, Revised June 16, 2010, Accepted for publication June 16, 2010

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Table 1. Results of studies on skin lesions in psoriasis patients

No.	Sex/Age (year)	Dur	Acute course	PASI	Clinical characteristics	Itch	Distribution/lesion characteristics	CD4/CD8 (%)	FOXP3 (%), skin		
									Papillary tip	Dermis	Epidermis
1	M/37	8 yr	N	3.0	Mild	±	Solitary	50/50	20~30	10	0
2	F/19	4 mo	N	4.5	Mild	±	Typical	50/50	40	20	<5
3	F/67	1 mo	N	5.0	Mild	+	Typical	80/20	40	15	0
4	F/6 mo	3 wk	Y	9.9	Sev	—	Annular, Koebner	40/60	<5	1~3	<5
5	M/82	10 yr	N	7.2	Mod	+	Typical	60/40	40	15~20	<5
6	M/65	6 mo	N	7.2	Mod	+	Typical	60/40	40~50	30	<5
7	F/25	3 wk	Y	0.6	Mod	—	Guttate	ND	<5	0	<5
8	M/65	10 mo	Y	2.0	Mod, Sev	++	Eczema	ND	10	10	<5
9	M/59	3 yr	N	5.9	Mild	±	Typical	50/50	30	20	<5
10	F/29	10 yr	N	4.5	Mild	—	Solitary	50/50	40	20	25
11	M/21	9 yr	N	12.6	Mod	—	Typical	50/50	40	10	40
12	F/20	3 yr	N	6.4	Mod	+	Typical	50/50	20~30	10	15
13	M/38	5 mo	N	5.6	Mild	±	Palm	ND	20~30	10	30
14	F/36	1 mo	Y	5.2	Mod, Sev	+	Guttate	ND	<5	<5	0
15	F/30	1 mo	Y	1.5	Mild	+	Palm	ND	20~30	10	20
16	M/5	2 wk	N	2.9	Mild	+	Typical	ND	20~30	5	<5
17	M/50	1 yr	N	1.8	Mild	±	Typical	ND	40	5	<5
18	M/35	3 yr	Y	35.9	Sev	+	Erythroderma	ND	0	<5	0
19	F/48	1 yr	Y	9.6	Mod	+	Guttate	ND	<5	10	0
20	F/45	3 mo	Y	9.7	Mod, Sev	+	Eczema, few pustule	ND	<5	<5	0
21	F/62	3 d	Y	6.2	Mod	+	Eczema	ND	20	10	<5
22	F/50	5 yr	Y	3.6	Mod	+	Guttate	ND	20	<5	<5

Dur: duration, PASI: psoriasis area and severity index, Sev: severe, Mod: moderate, ND: not done.

of Asan Medical Center. From April 2004 to June 2006, in the dermatology clinic of Asan Medical Center, 4-mm punch biopsies were taken from the untreated lesional skin of 22 patients with psoriasis (10 males, 12 females, mean age 41.0 years). In psoriasis patients, biopsies were taken from the center of the lesion. For controls, 5 skin biopsy samples were obtained from the normal skin of the abdomen (1 male, 4 females, mean age 33.2 years). Review of clinical records, medical photographs and biopsy slides was done and clinicopathologic correlation was performed by two independent dermatologists. In the psoriatic patients, psoriasis area and severity index (PASI) scores were recorded (Table 1).

For flow cytometry, peripheral venous blood was drawn from 18 other patients with mild to moderate psoriasis (10 males, 8 females, mean age 39.6 years, 6 acute course and 12 with chronic course) and 8 healthy volunteers (6 males, 2 females, mean age 26.9 years). From these, the available peripheral blood samples of 8 psoriasis patients (4 males, 4 females, mean age 45.2 years; 4 with acute course and 4 with chronic course) was used to perform reverse transcriptase polymerase chain reaction (RT-PCR).

Immunohistochemistry of the skin

We performed immunohistochemical stainings in formalin-fixed paraffin-embedded sections. Standard streptavi-

din-biotin peroxidase method was used. Primary antibodies included CD3 (Dako, Glostrup, Denmark), CD4 (Dako), CD8 (Dako), CD79 (Dako), and FOXP3 (Mouse IgG1, Abcam, Cambridge, UK). In brief, 5- μ m transverse sections of paraffin-embedded tissue were cut, deparaffinized and then immersed in 3% hydrogen peroxide in methanol for 30 minutes to quench endogenous peroxidase activity. Antigen retrieval was achieved by microwaving the slides for 18 minutes (8 minutes to boiling and 10 minutes at boiling) in 0.01 M citrate buffer (pH 6.0). Sections were incubated with the primary monoclonal antibodies mentioned above. After washing in Tris-buffered saline, pH 7.6, incubation with a secondary biotinylated antibody (Vectastain Universal Elite ABC kit; Vector Laboratories, Peterborough, England) was carried out for 30 minutes at room temperature. A tertiary peroxidase-labeled avidin-biotin complex was applied at room temperature for 30 minutes before developing with diaminobenzidine (Dako). All slides were counterstained with Hematoxylin (Dako) and dehydrated in a series of alcohols before being mounted with a nonaqueous mounting media. The results were evaluated by two dermatologists and one pathologist. Cells were counted in three fields per high power field (magnification \times 200).

Flow cytometry of blood

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood using density gradient centrifugation on Ficoll. The antibodies included CD3 PE (Dako), CD4 PerCP, CD8 FITC, CD25 FITC (all from BD-Pharmingen) and FOXP3 (Abcam). Appropriate isotype controls were included. Two-color flow cytometry was performed by incubation of 5×10^5 peripheral blood mononuclear cells with the indicated monoclonal antibodies (mAb). FOXP3⁺ cells or CD4⁺CD25^{high} cells were analyzed. Differences were analyzed using ANOVA and rank sum test and $p < 0.05$ was considered to be statistically significant.

RT-PCR for detection of FOXP3⁺ mRNA in peripheral blood

Total RNA was isolated from whole blood using RNeasy kit (Qiagen, Hilden, Germany) according to the manu-

facturer's instructions and reverse-transcribed. To exclude the possibility of DNA contamination, mRNA was treated with DNase (Qiagen). The PCR primers (Qiagen) were: 5'-ACACCACCCACCACCGCCACT-3' for forward *foxp3* and 5'-TCGGATGATGCCACAGATGAAGC-3' for reverse *foxp3*. Thermal cycling conditions were as follows: Initial template denaturation at 94°C for 5 minutes followed by primer annealing for 35 cycles of amplification at 57°C, extension at 72°C for 1 min and melting at 95°C for 1 min. PCR products were analyzed by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining.

RESULTS

The population of FOXP3⁺Treg in psoriatic skin lesions and in normal skin

In skin biopsy samples from the normal skin, the percentage of FOXP3⁺Tregs among the dermal mononuclear cells was about 10~20% (Fig. 1).

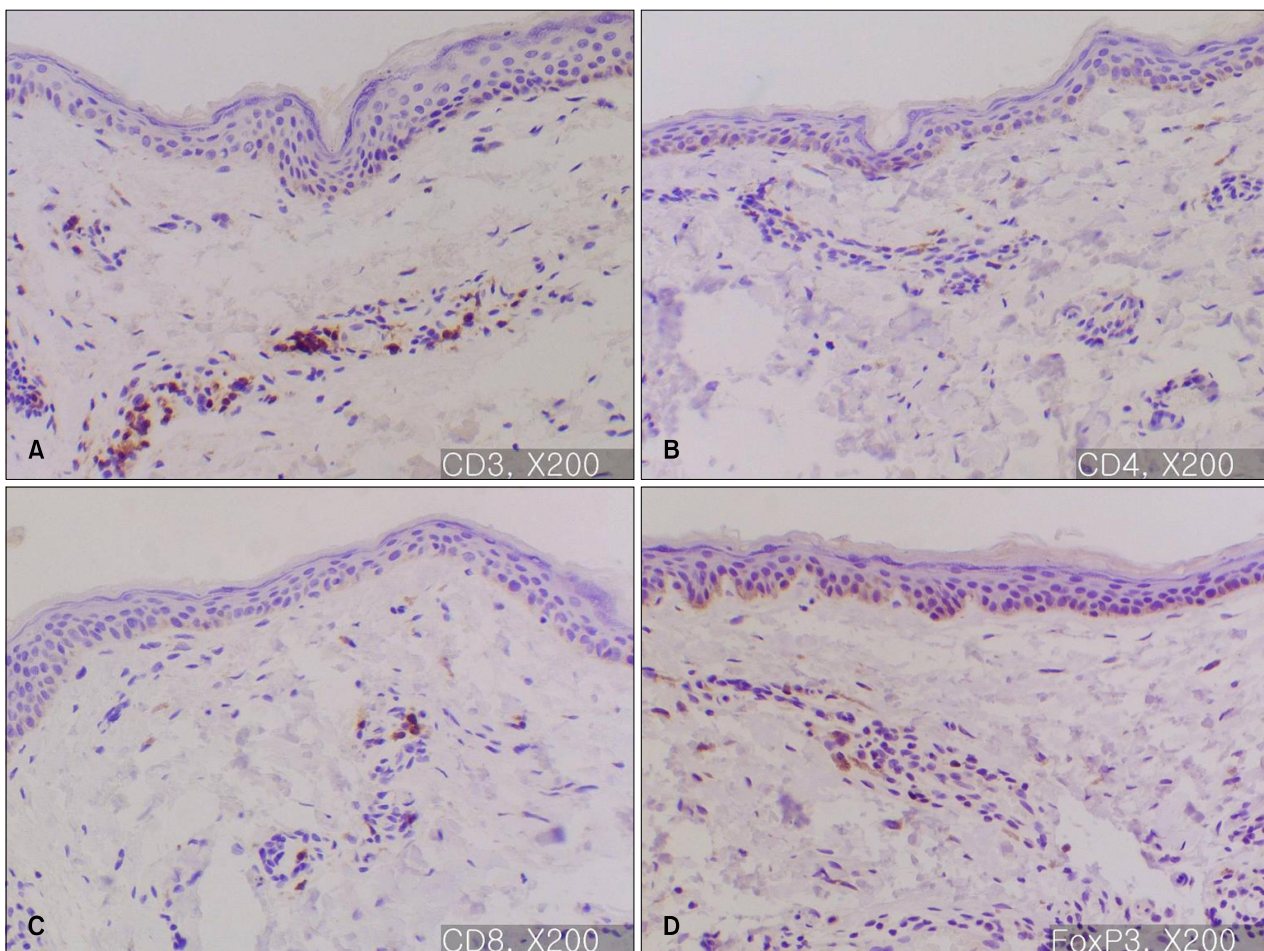


Fig. 1. Immunohistochemistry of normal skin (A: CD3, $\times 200$, B: CD4, $\times 200$, C: CD8, $\times 200$, D: FOXP3, $\times 200$). The percentage of FOXP3⁺ mononuclear cells in normal skin is 10~20%; the mononuclear cells are CD3, CD4 and CD8⁺.

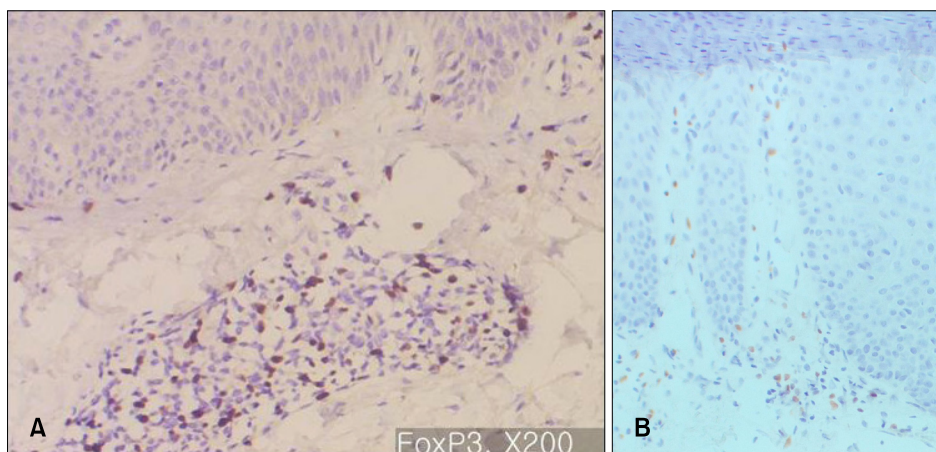


Fig. 2. (A) Immunohistochemistry for FOXP3, $\times 200$: Typical plaque type psoriasis (Case No. 3). In psoriatic skin lesions, FOXP3⁺ cells are mainly observed in papillary dermis, consisting of about 20~40% of the mononuclear cellular infiltration. (B) Immunohistochemistry for FOXP3, $\times 200$: Palmoplantar pustular psoriasis (Case No. 15). FOXP3⁺ cells consists of about 20~30% of the mononuclear cellular infiltration.

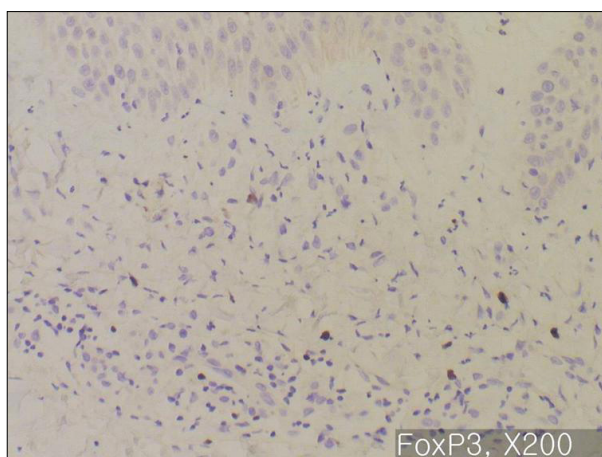


Fig. 3. Immunohistochemistry for FOXP3, $\times 200$: Acute severe plaque type psoriasis (Case No. 4). FOXP3⁺ T cells are relatively deficient.

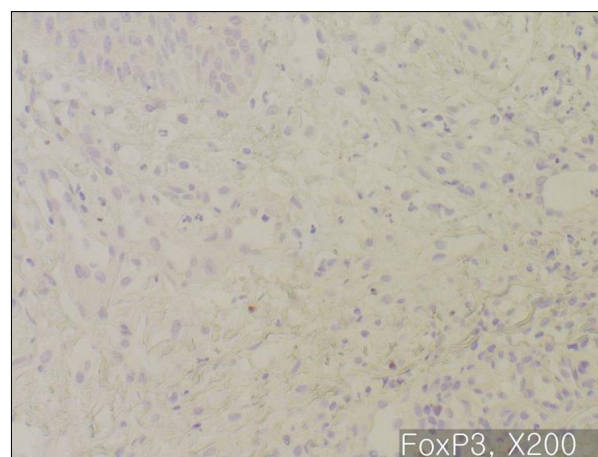


Fig. 5. Immunohistochemistry for FOXP3, $\times 200$: Acute exacerbating erythrodermic psoriasis (Case No. 18). FOXP3⁺ cells are almost absent.

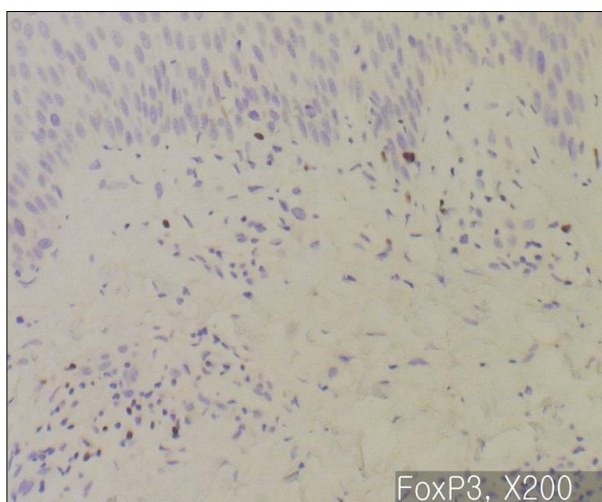


Fig. 4. Immunohistochemistry for FOXP3, $\times 200$: Acute moderate to severe plaque type psoriasis (Case No. 8). The ratio of FOXP3⁺ Treg to mononuclear cells is decreased.

In majority of the psoriatic skin lesions, FOXP3⁺ cells were mainly observed in the papillary dermis, consisting of about 20~40% of the mononuclear cellular infiltration (Fig. 2A). There was no difference in the population of FOXP3⁺Treg within solitary plaque (case No. 1, 10) and in palmoplantar pustular psoriasis (case No. 13, 15) as compared to that in plaque-type psoriasis (Fig. 2B). In seven psoriasis patients (31.8%), the percentage of FOXP3⁺ Tregs decreased to less than 10% of T cell infiltrates in the papillary dermis (Fig. 3~5). Due to initiation or acute exacerbation of psoriatic skin lesions in these patients at the time of skin biopsy, the clinical characteristics of these seven cases were categorized into the 'acute course'. They comprised of guttate type (n=3, case No. 7, 14 and 19 of Table 1), acute severe plaque type (n=1, case No. 4 of Table 1), acute erythrodermic type (n=1, case No. 18 of Table 1), and acute moderate to severe plaque type (n=2, case No. 8 and 20 of Table 1). Among these, in one case

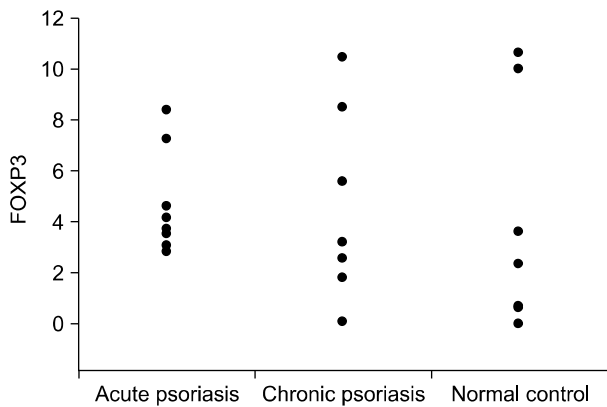


Fig. 6. Result of flow-cytometry for FOXP3. In all samples, the percentage of FOXP3⁺ was 3~10% among the peripheral blood mononuclear cells of the normal control group (n=8) and the results were similar between the cases of acute psoriasis (n=6), and chronic psoriasis (n=12).

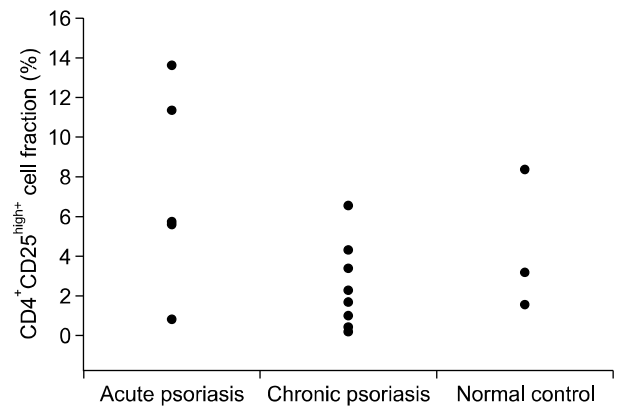


Fig. 7. Results of flow-cytometry for CD4 and CD25. CD4⁺CD25^{high+} cell fraction ranged between 3~10% of the peripheral blood mononuclear cell in all subsets of the patients.

of acute severe plaque type (case No. 4 of Table 1), in three cases of acute guttate type (case No. 7, 14 and 19 of Table 1), and in one case of acute erythrodermic type (case No. 18 of Table 1), the percentage of FOXP3⁺Tregs was less than 5% of the papillary dermal mononuclear cells.

RT-PCR and flow cytometry in peripheral blood of psoriasis patients and normal controls

In RT-PCR for FOXP3 mRNA in peripheral blood, no difference was observed between the cases with 'acute course' psoriasis (n=4), and the cases with 'chronic course' psoriasis (n=4) and the normal controls (n=5).

In flow cytometry, about 5% of FOXP3⁺ T cells were observed among the PBMCs of the normal control group (n=8) and the result was similar in the cases with 'acute course' psoriasis (n=6), and in the cases with 'chronic course' psoriasis (n=12). FOXP3⁺ or CD4⁺CD25^{high+} cell fraction ranged between 3~10% (Fig. 6, 7) of the PBMC in all the subsets of patients and no statistically significant difference was found in all the subsets of patients using the ANOVA and rank sum test ($p > 0.05$) (Fig. 6, 7).

DISCUSSION

'Regulatory T cells' are recognized as cells that have potent immune-regulatory function, especially the inhibition of T-cell activation and proliferation. Three subsets of regulatory T cells are discerned by surface molecule expression and mechanism of immunosuppression. These encompass Tregs, T-helper-3 cells (Th3), and type 1 regulatory T cells. Among these subsets, Tregs, the primary area of this study, are the most intriguing^{12,13}. On

their surface, they have the interleukin-2 receptor chain (CD25), which plays a vital role in maintenance of tolerance to self antigen and induction of non-responsiveness to alloantigen and prevents the activation and proliferation of auto- and allo-antigen-reactive T cells⁵. The role of Tregs in suppression of CD8⁺ T-cell activation as well as in activation of allergen-specific Th1 and Th2 cells has been widely reported¹⁴. Tregs are believed to act through interaction with other T-cells via both cell-to-cell contact mechanism and indirect cytokine-dependent mechanism¹⁴⁻¹⁶. FOXP3 gene is mutated in X-linked autoimmunity-allergic dysregulation syndrome in humans and in 'scurfy' mice. Overexpression of FOXP3 has been shown to lead to a hypoactive immune state suggesting that it is a central regulator of T cell activity. FOXP3 has significance for several reasons. Firstly, FOXP3 is more specific marker of Tregs than most cell surface markers such as CD4 and CD25. Secondly, FOXP3 plays an important role in the development of Tregs. It has been observed that the transduced expression of FOXP3 in CD4⁺CD25⁻ T cells has been shown to induce GITR, CD103 and CTLA-4, which are the surface molecules expressed on Tregs. Thirdly, the expression of FOXP3 reflects the activity of Tregs¹⁻⁴.

Our results indicate that the percentage of FOXP3⁺Tregs among T lymphocytes increased in the skin lesions of patients with chronic plaque type psoriasis, but the percentage decreased in the skin lesions of patients with psoriasis with acute course. It seems that it is not the absolute number of FOXP3⁺Tregs, but it is their relative proportion which is more important. The degree of inflammatory cell infiltrate in the lesional psoriatic skin varies, thus the increase in the absolute number of FOXP3⁺ Tregs may be the result of non-specific inflammation of unknown significance. This may explain the variable and

inconsistent results of previous studies on FOXP3⁺Treg population in the lesional psoriatic skin ranging from increment to total absence of FOXP3⁺Tregs. We suggest that lesional variation of FOXP3⁺Treg population according to biopsied sites in psoriatic skin should be considered in those studies, given that the localization of most FOXP3⁺Tregs in the center of the psoriatic plaque rather than in the periphery¹⁷. In the present study, we took skin biopsies from the center of the lesional psoriatic skin to avoid sampling error.

FOXP3 positivity can represent the activity of Tregs, but the possibility of reactive FOXP3⁺Treg increment with functional aberration should also be taken into consideration. Sugiyama et al.¹⁸ have reported that Tregs are numerically and functionally deficient in their ability to suppress the abnormally persistent psoriatic immune response, in both the lesional skin and in peripheral blood. Although psoriasis is considered to be a systemic disease with some immunologic features, alteration in the percentage of the population of FOXP3⁺Tregs in PBMC was not observed in the present study. Functional aberration in FOXP3⁺Tregs may explain the lack of difference between the acute psoriasis cases, the chronic psoriasis cases and the normal controls on flow cytometry of blood.

In psoriasis, the expression of specific transcription factor of Tregs, FOXP3, was found to be decreased or was aberrant in the results of previous studies^{18,19}. Sugiyama et al.¹⁸ have shown the deficiency and dysfunction of Tregs in the psoriatic plaques. In their study, the number of Tregs in peripheral blood of psoriasis patients was normal, but were dysfunctional¹⁸. In another study¹⁹, Tregs were detected in the psoriatic skin lesions but not in the normal skin. In psoriasis, while a majority of dermal T-cell infiltrates were CD4⁺ and most of the T-cells in epidermis were CD8⁺, Tregs were seen predominantly in the upper dermis of the psoriatic plaques.

In this study, size of the lesions, itching and duration of the skin lesions had no effect on the FOXP3⁺Treg levels (Table 1). Also, neither the extent nor the severity of the disease were related to the FOXP3⁺Treg levels. Rather, FOXP3⁺Tregs were reduced in the lesional skin of acute onset psoriasis. Based on this finding, we can suggest that FOXP3⁺Tregs play a key role in acute exacerbation when the balance of immunosuppression and immune activation is just about to shift, but not in stable maintenance. It could be also inferred that the increase in Tregs in chronic plaque type psoriasis might be compensatory to the immune activation, and the consequential immunosuppression might contribute to the immune balance throughout the course of the disease.

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